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Protein kinase C beta (PKC beta): normal functions and diseases.
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Nakashima S.
Protein kinase C alpha (PKC alpha): regulation and biological function.
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# Protein Kinase Cβ (PKCβ): Nomal Functions and Dieases

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**PKCβI and PKCβII are DAG- and $Ca^{2+}$-dependent conventional or classical isoforms of protein kinase C. Generated by alternative splicing from a single gene, they differ at their C-terminal 50 (βI) or 52 (βII) residues. They are expressed as major PKC isoforms in a variety of tissues, and thus the functions ascribed to "PKC" based on early studies using phorbol esters and PKC inhibitors could be attributed to them. As tools to probe into isoform-specific functions have recently become available, our understanding of the normal functions of these isoforms has dramatically increased. This minireview will focus mainly on two areas of signal transduction where the roles of PKCβI and PKCβII are relatively well-characterized: immunoreceptor and insulin receptor systems. Their involvement in disorders due to pertubations in these signaling systems, *i.e.*, immunodeficiencies and diabetes, is also reviewed. Finally, patterns of PKC action in these and other biologic systems are discussed.**

**Key words: BCR, diabetes, FcεRI, insulin, PKCβ.**

A single gene locus (*PKCβ*) encodes two proteins, PKCβI and PKCβII, which are generated by alternative splicing of the C-terminal exons (*1*). Thus, the difference between these two isoforms resides in the C-terminal V5 domains, which still exhibit a moderate homology (45%) at their amino acid sequences (Fig. 1). Both PKCβI and PKCβII are classified as conventional or classical isoforms whose optimal activity requires diacylglycerol (DAG) and $Ca^{2+}$. They are expressed as major PKC isoforms in a variety of tissues and therefore the functions associated with "PKC" based on early experiments using PKC-activating phorbol esters and general PKC inhibitors could be attributed to them. Thus, PKCβI and βII might function in various signal transducing pathways for proliferation, differentiation, metabolism, and more cell-type-specific functions. Since Nishizuka wrote his landmark review a decade ago after the cloning of most of the currently known PKC isoforms (*2*), the PKC community has accumulated data on specific functions of PKCβI and βII, using isoform-specific cDNAs (wild-type [wt], constitutively active, and dominant negative [DN]), antisense oligonucleotides, PKCβ-specific inhibitors, transgenic mice, and gene knockout mice. This review will focus on recent developments in two areas that illustrate the specific roles of these PKC isoforms and the principles in their signaling networks with regard to other molecules. Readers are referred to other reviews in this series as well as those by Newton and Mochly-Rosen for activation mechanisms and adaptor or scaffold proteins (*3–5*).

## Immunoreceptor signaling and immunodeficiency

Stimulation of antigen or Fc receptors on immune cells such as the B cell receptor (BCR) on B lymphocytes and the high-affinity IgE receptor (FcεRI) on mast cells triggers a concerted activation of Src, Syk, and Tec family protein-tyrosine kinases (PTKs). These receptor-proximal PTKs in turn activate several signaling pathways including phosphatidylinositol 3-kinase (PI3K), phospholipase C (PLC)-γ, and $Ca^{2+}$. Products of PLC activity, *i.e.*, DAG and inositol 1,4,5-trisphosphate (IP3), together with consequent increases in $Ca^{2+}$, can activate conventional and novel isoforms of PKC. As can be seen from the fact that phorbol ester plus $Ca^{2+}$ ionophore can circumvent the requirement for receptor stimulation to activate the immune cells, PKC and $Ca^{2+}$ play critical roles in immune cell activation. Indeed, studies on *PKCβ* gene knockout mice showed that PKCβ is critically important in B cell development and activation (*6*). These mice had fewer splenic B cells than normal, drastically reduced B-1 lymphocytes and low levels of serum IgM and IgG3, and mounted defective immune responses to thymus-independent type II antigen and reduced primary responses to T cell–dependent antigen. B cell proliferative responses to BCR and lipopolysaccharide stimulation were also reduced. In contrast, T cell development and proliferation in response to CD3 stimulation were normal in *PKCβ −/−* mice, although PKCβ was expressed in T cells. The B cell phenotype of these mice was similar to those of *btk* knockout mice and X-linked immunodeficient (*xid*) mice with a missense (R28C) mutation in Btk (*7*), suggesting a signaling link between PKCβ and Btk. Indeed, Btk physically interacts with various isoforms of PKC *in vitro* through the interaction between the PH domain of Btk and the C1 domain of PKC (*8, 9*) and co-immunoprecipitates with PKCβI from mast cell lysates

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Abbreviations: BCR, B cell receptor; DAG, diacylglycerol; DN, dominant negative; EGF, epidermal growth factor; Egr, Early growth response; FcεRI, high-affinity IgE receptor; IKK, IκB kinase; IP3, inositol 1,4,5-trisphosphate; IRS, insulin receptor substrate; LSP1, leukocyte-specific protein-1; PI3K, phosphatidylinositol 3-kinase; PMA, phorbol 12-myristate 13-acetate; PLC, phospholipase C; PTK, protein-tyrosine kinase; Rb, retinoblastoma; VEGF, vascular endothelial growth factor; wt, wild-type; xid, X-linked immunodeficient.

(*10*). PKCβ (either βI or βII) can phosphorylate Ser180 in the Tec linker region of Btk and inhibits the membrane translocation and tyrosine phosphorylation at Tyr551 and Tyr223 of Btk (*11*). Consistent with these observations, Btk phosphorylation was increased in BCR-stimulated PKCβ −/− cells (*6*). On the other hand, Btk in concert with Syk phosphorylates and regulates the activity of PLC-γ2 (*12*), and, apparently through this mechanism, Btk positively regulates the membrane translocation and activity of PKCβI, not PKCβII or PKCα, in FcεRI-stimulated mast cells (*10*). This Btk/PKCβI pathway is involved in FcεRI-induced production of IL-2 and TNF-α. PKCβI was also shown to be required for IL-2 secretion from PMA-stimulated T cells (*13*). FcεRI stimulation induced a reduced production of IL-6 mRNA and protein in PKCβ −/− mast cells (*14*), which is consistent with an increased accumulation of IL-6 mRNA in PKCβ-overexpressing mast cells (*15*). Since PKCβ (and PKCε) can be involved in FcεRI-induced expression of c-*fos* and c-*jun* mRNAs (*16*), reduced induction of JunD mRNA may contribute to the reduced IL-6 production in *PKCβ* −/− mast cells. Collectively, not only does Btk positively regulate PKCβI, but PKCβ can negatively regulates Btk activity as a feedback loop inhibitor (Fig. 2). This self-regulatory pathway may contribute to fine-tuning of signal intensity and timing. The importance of this kind of fine-tuning was illustrated in B cell immunodeficiencies because loss-of-function as well as gain-of-function mutations of Btk in-duce similar immunodeficiencies (*7*). The phenotypic similarity of *PKCβ* −/− and *btk* −/− (or *xid*) mice may be understood along this line.

An important outcome of immunoreceptor activation is the activation of NF-κB, which is involved in immune reactions, inflammation, and cell survival. NF-κB is a heterodimer of p50 and p65 that is sequestered in the cytosol by IκB, which prevents its nuclear translocation and activity. Upon immune cell activation, IκBα is phosphorylated by IκB kinases (IKKα and IKKβ) of the IKK complex, which triggers the ubiquitination and subsequent degradation of IκB through the proteasome pathway. Phosphorylation of IκBα downstream of BCR triggering is regulated by Btk (*17, 18*). Defective IκBα phosphorylation and inefficient Bcl-$X_L$ induction in *btk* −/− or *xid* B cells indicate an important role of Btk-mediated NF-κB activation in BCR-dependent B cell survival. PKCβ was also shown to control NF-κB activity (*19, 20*). Similar to *btk* −/− B cells, *PKCβ* −/− B cells are also characterized by poor survival in the absence of IL-4. Consistent with their poor proliferative response, BCR stimulation failed to promote the expression of the anti-apoptotic proteins Bcl-$X_L$ and Bcl-2 in *PKCβ* −/− B cells. In these cells, BCR stimulation also failed to induce the recruitment of the IKK complex to lipid rafts (*19*) and phosphorylation of IKKα at the critical Ser180 and sustained Ser181 phosphorylation of IKKβ (*20*), which resulted in reduced phosphorylation and more persistent expression of IκBα, leading to a reduced NF-κB activity. However, the reduction of NF-κB activity was only modest (*20*), which is potentially congruent with the study that indicates that novel PKC isoforms (particularly PKCθ and

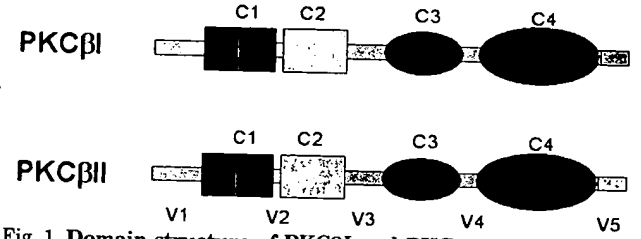


Fig. 1. **Domain structure of PKCβI and PKCβII.** The DAG-binding C1 domain, the acidic phospholipid/$Ca^{2+}$-binding C2 domain and the catalytic domain (C3-V4-C4) are highlighted. The V5 region is different between PKCβI (50 resides) and PKCβII (52 residues), but key autophosphorylation sites (Thr641 and Ser660 in PKCβII) in this region are conserved.

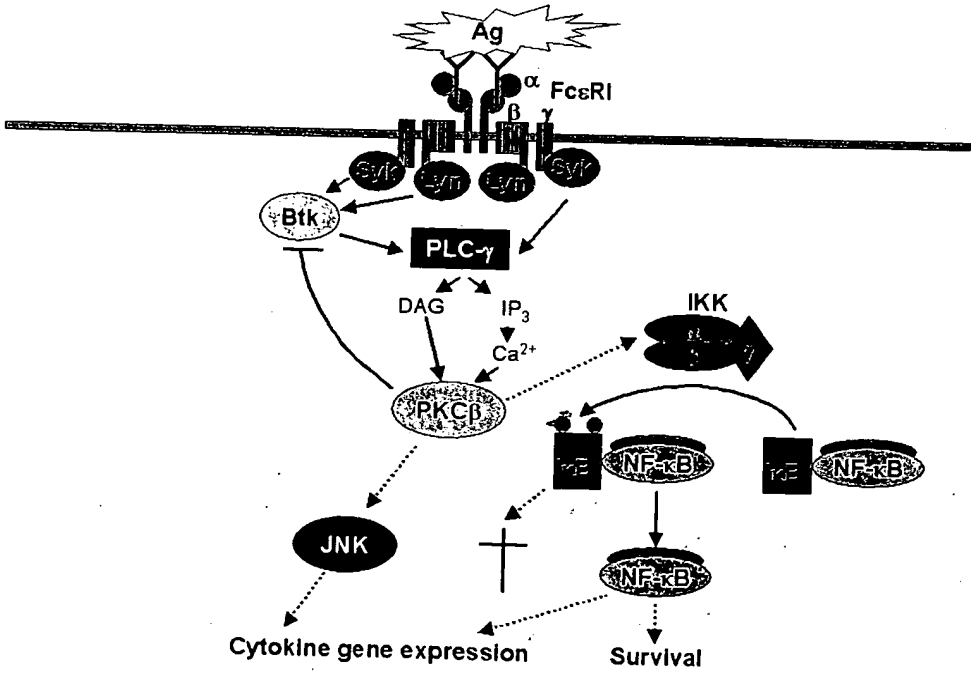


Fig. 2. **PKCβ in immunoreceptor signaling.** Btk phosphorylates and activates PLC-γ in concert with Syk upon immunoreceptor stimulation. Second messengers generated by PLC, *i.e.*, DAG and IP3 (subsequent $Ca^{2+}$ increase), activate various PKC isoforms including the βI isoform. Activated PKCβI can phosphorylate Ser180 of Btk and inhibit the activity of the latter enzyme. PKCβ can also regulates transcription of several cytokine and survival genes such as *bcL-$X_L$* through JNK and IKK activities. The simplified signaling pathways of the FcεRI system are depicted. BCR signaling also uses similar signaling networks.

PKCδ), but not PKCβII, seem to play a critical role in BCR-mediated NF-κB and JNK activation (*21*). Importantly, PKCβ inhibitors blocked survival of cell lines derived from non-Hodgkin's diffuse large B cell lymphomas (*19*), suggesting a clinical potential of PKCβ inhibitors in treating these B cell tumors. The importance of PKCβI in B cell survival was also shown in a subline of WEHI-231 cells that express C-terminal residues 179–330 of leukocyte-specific protein 1 (LSP1) (*22*). This LSP1 truncate, termed B-LSP1, inhibited anti-IgM–induced membrane translocation of PKCβI, but not PKCβII or PKCα, and ERK2 activation, and increased anti-IgM–induced apoptosis. Inhibition of ERK2 activation contributes to the increased apoptotsis. Although B-LSP1 can directly interact with PKCβI, but not PKCβII or PKCα, it is not clear whether the endogenous LSP1 protein plays the same role as a PKCβI-sequestering protein in BCR-mediated apoptosis. However, the role of PKCβI in survival could be cell-type-specific; no adverse effects of the lack of PKCβ were observed on mast cell proliferation and survival (*14*).

Degranulation is a cardinal feature of FcεRI-induced mast cell activation that requires both PKC and $Ca^{2+}$ for maximal activity. Early studies implicated PKCβ and PKCδ in this function based on the reconstitution of degranulatory activity in permeabilized and PKC-depleted mast cells by incubation with individual recombinant PKC isoforms (*23*). Indeed, *PKCβ* −/− mast cells exhibited a lower degranulatory activity than wt cells in response to FcεRI or $Ca^{2+}$ ionophore stimulation (*14*). However, *PKCδ* −/− mast cells showed a higher degranulatory activity, particularly when IgE-primed cells were stimulated with high concentrations of antigen (*24*). *PKCε* −/− and *PKCθ* −/− mast cells degranulated indistinguishably from wild-type cells (our unpublished data).

### Insulin receptor signaling and diabetes

Like other receptor PTKs such as those for epidermal growth factor (EGF) and platelet-derived growth factor, stimulation of insulin receptor also activates PLC and production of DAG, which in turn activates several PKC isoforms. PKCs may activate Ras and the Raf/MEK/ERK pathway, while DAG-responsive PKCs may activate Raf in a Ras-independent manner. In L6 skeletal muscle cells, insulin-induced activation of PKCα, PKCβ, ERK1/2, and DNA synthesis was largely dependent on phosphorylation of insulin receptor substrate 1 (IRS-1), not IRS-2. Blocking PKCβ (not PKCα) with either antisense oligonucleotide or the PKCβ-specific inhibitor LY379196 decreased the insulin-induced ERK activity and DNA synthesis, without affecting EGF- or serum-stimulated mitogenesis. In contrast, the inhibition of Ras largely spared insulin-induced ERK activation and DNA synthesis, whereas it blocked EGF-induced ERK activation and mitogenesis. PKCβ blockade did not affect Ras activity but inhibited insulin-induced Raf activation and coprecipitation of Raf with PKCβ. Based on these observations, a signaling pathway was proposed for PKCβ-mediated regulation of the Raf/MEK/ERK module and mitogenesis (Fig. 3) (*25*). Interestingly, PKCζ plays a major role in insulin induction of ERK activity in rat adipocytes (*26*).

In addition to the positive role of PKCβ in insulin receptor signaling, activation of PKC is associated with an inhibition of insulin receptor PTK activity in various cell types. Downregulation of insulin receptor kinase activity contributes to the pathogenesis of cellular insulin resistance in diabetes mellitus. When the human insulin receptor was coexpressed with PKCβI and βII isoforms and stimulated by insulin in the presence of phorbol ester in HEK293 cells, tyrosine autophosphorylation of the insulin receptor was inhibited, while coexpression with the other isoforms did not significantly modify receptor autophosphorylation, suggesting that PKCβI and βII isoforms might be candidates for insulin receptor inhibition (*27*). However, similar overexpression of PKC isoforms in Chinese hamster ovary cells did not affect insulin-stimulated tyrosine phosphorylation of the receptor or its kinase activity. PKCα, but not βI, γ, or ε isoforms, inhibited in vivo insulin receptor kinase activity (*28*). Exposure to high glucose in L6 cells induced the acti-

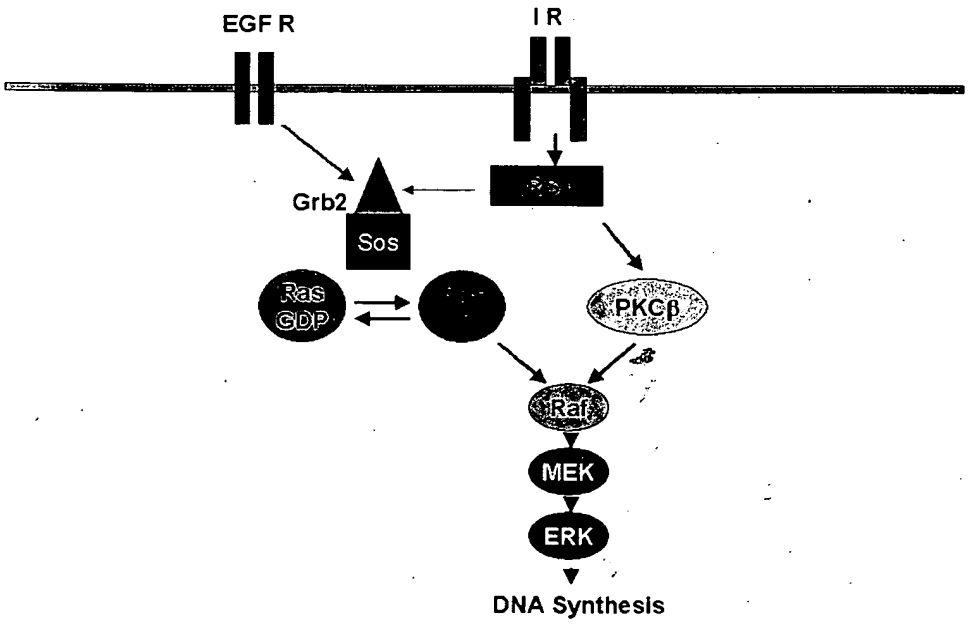


Fig. 3. **PKCβ in insulin signaling.** Insulin-stimulated insulin receptor (IR) phosphorylates IRS1. IRS1 recruit various signaling molecules through SH2-phosphotyrosine interactions. IRS1-binding proteins containing an SH2 domain include Grb2, PI3K, SHP-2, and Nck. Insulin-induced ERK activation in L6 myocytes occurs largely through Raf-1 rather than Ras activation. In contrast, EGF-induced ERK activation is dependent on Grb2/Sos-mediated Ras activation.

vation of insulin receptor kinase activity as well as membrane translocation of glucose transporters, GLUT1 and GLUT4, and glucose uptake. These glucose effects were paralleled by a decrease in receptor-associated PKC activity, which was mostly accounted for by dissociation of PKCα, but not of PKCβ or δ, from the receptor. Thus, glucose autoregulation appears to involve PKCα dissociation from the insulin receptor (*29*). Interesting is the finding that glucose transport is increased in some tissues in *PKCβ* −/− mice and that the increased glucose transport may be partly due to the loss of PKCβI, which negatively modulates insulin-stimulated GLUT4 translocation (*30*).

Early studies indicated that PKC activity is increased in the retina, aorta, heart, and renal glomeruli of diabetic animals, probably because of an increase in de novo synthesis of DAG induced by hyperglycemia. PKCβII was shown to be preferentially increased in membrane fractions in the aorta, heart, and glomeruli of diabetic rats (*31*). The involvement of PKCβII in the development of retinopathy and nephropathy in diabetes was strongly suggested by experiments using a specific PKCβ inhibitor: LY333531 ($IC_{50}$ of 4.7 and 5.9 nM for βI and βII, respectively) inhibited PKC activity in the retina and glomeruli, and ameliorated the glomerular filtration rate, albumin excretion rate, and retinal circulation in streptozotocin-induced diabetic rats in an oral dose-responsive manner (*32*). On the other hand, overexpression of PKCβII in the myocardium in transgenic mice induced left ventricular hypertrophy, cardiac myocyte necrosis, multifocal fibrosis and decreased left ventricular performance. The severity of the phenotype was gene dosage–dependent, and this cardiovascular disease was largely prevented or reversed by LY333531 treatment (*33*).

Hyperglycemia is associated with the reduced expression of many islet β-cell–associated genes including the insulin gene. However, c-*myc* expression is induced in diabetic states. Among PKC isoforms (α, βII, δ, ε, and ζ) expressed in rat pancreatic islets, wt and DN mutant of PKCβII, but not other isoforms, influenced c-*myc* expression: wt PKCβII increased c-*myc* expression, and c-*myc* induction by high glucose was suppressed by DN PKCβII (*34*). Further, overexpression of wt PKCβII led to suppression of insulin gene transcription.

Neovascularization is involved in various diseases such as proliferative diabetic retinopathy, tumor growth, and rheumatoid arthritis. Vascular endothelial growth factor (VEGF) plays a central role in the development of neovascularization. Angiogenic response to oxygen-induced retinal ischemia was dramatically increased in transgenic mice overexpressing PKCβII and significantly reduced in *PKCβ* −/− mice (*35*). The mitogenic action and ERK1/2 activation by VEGF, a potent hypoxia-induced angiogenic factor, was increased in retinal endothelial cells by the overexpression of wt PKCβI or βII iosforms and inhibited by the expression of DN PKCβII. PKCβII was also shown to be physically asociated with retinoblastoma (Rb) protein and to phosphorylate the latter at specific serine residues. These observations suggest that Rb phosphorylation by PKCβ could lead to an increased transcriptional activity of E2F and eventually to increased VEGF-induced endothelial cell proliferation (*35*). PKCβ activation is also involved in fibrin deposition in hypoxemic vasculature by inducing tissue factor. Hypoxia induces membrane translocation and autophosphorylation of PKCβII, but not α or ε isoforms, in U937 monocytic cells (*36*). Tissue factor expression in an oxgen-deficient environment is driven by the transcription factor Early growth response (Egr)–1. *PKCβ* −/− mice exhibited markedly blunted tissue factor and vascular fibrin deposition responses when exposed to hypoxia. Consistent with the role of Egr-1 in these responses, the mutant mice displayed only a minor elevation of Egr-1 mRNA, protein and activity. PKCβ mediates the activation of ERK1/2, which in turn activates the transcription factor Elk-1. Elk-1, in complex with serum response factor, is the likely proximal trigger of Egr-1 transcription (*36*).

## Concluding remarks

PKCβI and βII play crucial roles in numerous cellular functions. Unfortunately, the mechanisms for these functions are still not completely understood. In particular, identification of direct phosphorylation targets and roles of associated proteins are lacking in most cases. However, some features in PKCβ action have emerged. First, a single PKC isoform exerts apparently positive as well as negative effects along the same signaling pathway (*e.g.*, PKCβI in BCR and FcεRI signaling). Second, different isoforms play the same role depending on cell types (*e.g.*, PKCβ and novel PKCs in BCR-mediated NF-κB activation and atypical PKCs in TNF-α–mediated NF-κB activation, PKCβ in myocytes and PKCζ in adipocytes for insulin induction of ERK activation). Third, different isoforms sometimes play opposing roles in a cellular function [*e.g.*, positive and negative roles of PKCβ and PKCδ, respectively, in FcεRI-induced degranulation, and cardioprotective action of PKCε and cardiodamaging action of PKCδ (*37*)]. These features of PKC action seem to contribute to fine-tuning of signal transduction. However, they could be an obstacle to overcome when inhibitors and activators of PKC isoforms are used as therapeutics. Careful study at preclinical and clinical stages will be able to monitor potential unwanted side-effects. Importantly, several hopeful reports of PKCβ inhibitors in preclinical and clinical settings (*32, 38*) are encouraging the PKC research community and pharmaceutical industry alike.

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